and purity. Sterile bacitracin zinc is the zinc salt of a kind of bacitracin or a mixture of two or more such salts. It is so purified and dried that:

(i) It contains not less than 40 units of bacitracin per milligram.

(ii) It is sterile.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) Its pH is not less than 6.0 and not more than 7.5.

(vi) Its zinc content is not more than 10 percent by weight on a moisture-free basis.

(vii) It passes the identity test.

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, each package shall bear on the outside wrapper or container and the immediate container the statement ''For use in the manufacture of topical drugs only''.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, loss on drying, pH, zinc content, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: Six packages, each containing approximately 1.0 gram.

(*b*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for bacitracin in §436.105 of this chapter, except add to each standard response line concentration sufficient 0.01*N* hydrochloric acid to yield the same ratio of 0.01*N* hydrochloric acid to 1 percent potassium phosphate buffer, pH 6.0 (solution 1) as present in the sample solution diluted to the reference concentration. Prepare the sample for assay as follows: Dissolve an accurately weighed sample (usually 25 to 35 milligrams) in sufficient 0.01*N* hydrochloric acid to give a bacitracin concentration of 100 units per milliliter (estimated). Further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid F in lieu of diluting fluid A.

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using a saturated solution (approximately 100 milligrams of the sample per milliliter).

(6) *Zinc content.* Proceed as directed in §436.312 of this chapter.

(7) *Identity.* Proceed as directed in §436.319 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 40 FR 15088, Apr. 4, 1975; 40 FR 19194, May 2, 1975; 42 FR 27230, May 27, 1977; 50 FR 19920, May 13, 1985]

§448.15a Sterile capreomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile capreomycin sulfate is the amorphous sulfate salt of capreomycin. It is a white or essentially white powder. Capreomycin has been separated chromatographically into components designated capreomycins Ia, Ib, IIa, and IIb. Each component has been partially characterized according to its type and amino acid content. Capreomycin Ia contains serine and no alanine. Capreomycin Ib contains alanine and no serine. Capreomycin I is a mixture of capreomycins Ia and Ib. It is so purified and dried that:

(i) Its potency is not less than 700 micrograms and not more than 1,050 micrograms of capreomycin per milligram on an ''as is'' basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of capreomycin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) It contains no depressor substance.

(vi) Its loss on drying is not more than 10 percent.

(vii) Its pH in an aqueous solution containing 30 milligrams per milliliter (or if packaged for dispensing, after reconstitution as directed in the labeling) is not less than 4.5 and not more than 7.5.

(viii) Its capreomycin I content is not less than 90 percent of the total capreomycins.

(ix) Its residue on ignition is not more than 3 percent.

(x) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, capreomycin I content, residue on ignition, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 100 micrograms of capreomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams per milliliter.

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter; however, if it is packaged for dispensing, use the solution obtained after reconstituting the drug as directed in the labeling.

(8) *Capreomycin I content*—(i) *Equipment*—(*a*) *Sheet* (*chromatographic*). Whatman No. 1 filter paper for chromatography 20 × 50 centimeters.

(*b*) *Chamber* (*chromatographic*). Square glass chromatography jar, 30 × 30 × 60 centimeters, designed for descending chromatography. The bottom of the tank is filled with 1.5 inches of a mixture of 70 percent *n*- propyl alcohol and 30 percent distilled water (v/v) and allowed to equilibrate for 2 days. The mobility of the capreomycin factors, capreomycin I and capreomycin II, depends to a large extent upon the amount of water vapor present in the chromatographic chamber. The mobility can be restricted by using more *n*-propyl alcohol and less water in the equilibrating solvent, or it can be increased by raising the water content. The R_f of value (the distance traveled by a particular antibiotic factor divided by the distance traveled by the solvent front) should be approximately 0.50 for capreomycin I and approximately 0.60 for capreomycin II.

(ii) *Preparation of solutions*—(*a*) *0.1N citrate buffer, pH 6.2.* Dissolve 21.0 grams of citric acid monohydrate in 1 liter of distilled water. Adjust the pH to 6.2 with 50 percent aqueous sodium hydroxide.

(*b*) *Developing solvent.* Mix *n*- propyl alcohol, distilled water, triethylamine, and glacial acetic acid in volumetric proportions of 75:33:8:8, respectively.

(iii) *Preparation of the capreomycin sample solution.* Dissolve approximately 200 milligrams of the sample, accurately weighed, with distilled water in a 10-milliliter volumetric flask. Dilute to volume with distilled water. This sample should be refrigerated when not in use.

(iv) *Preparation of the chromatogram.* Use separate sheets for each capreomycin sample solution and for blanks without sample application. Evenly apply a 100-microliter aliquot of the capreomycin sample solution to the origin line of a sheet. A U-shaped glass rod is placed under the chromatogram during spotting. Dry the streak thoroughly with warm air. Place the sample sheets and a blank sheet in the chamber and develop them in a descending manner for 16 hours. Remove the sheets from the chamber and air dry for about 1 hour.

(v) *Processing the chromatogram.* Examine each sheet under short-wavelength (254 nanometers) ultraviolet light and locate the main streak (R_f approximately 0.5) and the preceding streak (capreomycin II, R_f approximately 0.6). Outline the main zone lightly with a pencil. Outline an area on the blank sheet approximately equal in size and in the same location as those outlined on the sample sheets. Cut the marked areas from the sheets and then cut them into approximately 1.5-centimeter squares. For each sheet, place the squares into a glass-stoppered 50-milliliter Erlenmeyer flask.

(vi) *Elution.* To each flask, add 10 milliliters of 0.1*N* citrate buffer, pH 6.2, and agitate on a reciprocating shaker for 1 hour. Filter each of the shaken solutions through Whatman No. 1 filter paper into separate 10-milliliter glass-stoppered Erlenmeyer flasks. Transfer 3 milliliters of each filtrate into separate 50-milliliter volumetric flasks and dilute to volume with distilled water.

(vii) *Capreomycin sample solution for direct measurement of absorbance.* Pipette 1.0 milliliter of the sample solution prepared as described in paragraph (b)(8)(iii) of this section into a 100-milliliter glass-stoppered volumetric flask. Dilute to volume with 0.1*N* citrate buffer, pH 6.2. Transfer 3.0 milliliters of this solution into a 50-milliliter volumetric flask and dilute to volume with distilled water.

(viii) *Absorbance measurement.* Using a suitable spectrophotometer, 1.0-centimeter quartz cells, and distilled water as the reference solvent, determine the absorbance of each eluate and of each sample solution at the absorption maximum at about 268 nanometers.

(ix) *Calculation of percent capreomycin I in samples.* Calculate as follows:

$$\text{Percent capreomycin I} = \frac{A_I - A_B}{A_s} \times 100$$

where:

A_I=Absorbance of the eluate from the main zone of the sample sheet;

A_B=Absorbance of the eluate from the area of the blank sheet corresponding to the area of the capreomycin I of the sample sheet;

A_s=Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

If the assay of capreomycin I from the chromatogram is less than 90 percent of total capreomycins, repeat the procedure described in paragraph (b)(8)(iv), (v), (vi), (vii), and (viii) of this section two more times and at the same time determine the recovery of total capreomycins from the unchromatographed sheet as described in paragraph (b)(8)(x) of this section. The average of three valid assays should then be reported.

(x) *Recovery of total capreomycins from the unchromatographed sheet*—(*a*) *Procedure.* Evenly apply a 100-microliter aliquot of the capreomycin sample solution (prepared as described in paragraph (b)(8)(iii) of this section) to the origin line of a sheet. Dry the streak thoroughly with warm air. The paper is not chromatographed before elution. Cut the area containing the streak from the sheet and then cut into approximately 1.5-centimeter squares. Place the squares into a glass-stoppered 50-milliliter Erlenmeyer flask and proceed as directed in paragraph (b)(8)(vi), (vii), and (viii) of this section. Likewise, cut an equal-sized area from an untreated part of the sheet and cut it into approximately 1.5-centimeter squares. Place the squares

in a glass-stoppered 50-milliliter Erlenmeyer flask and also proceed as directed in paragraph (b)(8) (vi), (vii), and (viii) of this section.

(*b*) *Calculation.* Calculate the recovery of total capreomycins as follows:

$$\text{Recovery of total capreomycins} = \frac{At - Ab}{A_s} \times 100$$

where:

At=Absorbance of the eluate from the unchromatographed sheet;

Ab=Absorbance of the eluate from the unchromatographed blank sheet;

As=Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

To be a valid assay, the recovery of total capreomycins from the unchromatographed sheet must be 100±2 percent.

(9) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter, except ignite at 700° C.

(10) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 448.20a Sterile colistimethate sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistimethate sodium is the sodium salt of a kind of colistin methane sulfonate or a mixture of two or more such salts. It is a white to slightly yellow, odorless, fine powder which is freely soluble in water. It is so purified and dried that:

(i) Its potency is not less than 390 micrograms of colistin base equivalent per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of colistin base equivalent that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) It gives a positive identity test for colistimethate sodium.

(viii) It passes the test for free colistin.

(ix) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, identity, free colistin, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 containers, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 vials or if each vial contains less than 150 milligrams of colistimethate, a minimum of 60 vials.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: If the batch is packaged for repacking or for use in manufacturing another drug, dissolve an accurately weighed sample in 2 milliliters of sterile distilled water and further dilute with sufficient 10-percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. If it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if the container is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute the